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INIISOLATION AND IDENTIFICATION OF FUNGI FROM SAMPLES RECEIVED IN MICROBIOLOGY LAB FROM SKIN AND ENT OUTPATIENT DEPARTMENT OF A TERTIARY CARE HOSPITAL IN SOUTH INDIA

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ABSTRACT

Aim

To isolate and identify fungi from samples received in microbiology lab from DERMATOLOGY and ENT outpatient department of a tertiary care hospital.

Objectives

To isolate and identify the fungal strains by phenotypic methods from patients suffering with mycostic fungal infections from skin and ENT outpatient department of a tertiary care hospital

Background

One of the most common fungal disease in the world is superficial mycotic infections. Reliable data from the spectrum of pathogens causing fungal infections in various body systems particularly the antifungal susceptibility pattern of candida SPP which is the most common isolate worldwide. Candida isolate were differentiated into candida albicans and non candida albicans by the germ tube test.

Methods

The study population includes 100 patients with clinically suspected superficial fungal infections, who attend the out patient department of dermatology at Sree Balaji medical College and hospital, Chennai for a period of 4-6months . Skin scrapings, hair and nail samples were collected from patients with cutaneous infections. Direct wet mount microscopic examination was done using 20% potassium hydroxide (KOH) for nail and hair and 10% potassium hydroxide wet mount for skin scrappings. The collected specimens were inoculated into two sets of sabouraud's dextrose agar. The culture tubes were incubated at two different temperature 28% and 37degree c. After incubation, culture growth was observed once every two days and the tubes were discarded only after six weeks in the absence of growth. The Microscopic examinations of fungal growth were identified by lactophenol cotton blue stain.

Culture identifications is done based on growth rate, temperature, colony characteristics, colour, texture and pigment production, dermatophytes were identified depending on nature of mycelium and formation of macro and microconidia of the isolates, budding yeast cells of candida albicans and other candida species were identified by gram stain and LCB mount. Germ tube test was done for the identification of candida albicans.

RESULTS

The study population which included 100 patients with clinically suspected superficial fungal infections was subjected to mycological examination. Out of 100 specimens the KOH wet mount was positive for fungal elements in 70(70%) samples and culture positivity was 60(60%). Among these culture positive isolates 49(49%) were non dermatophytes and 11(11%) were dermatophytes, 40(40%) not grown in culture. Among the culture positive 49 non dermatophytes and 11 dermatophytic fungus which includes 12 Aspergillus Niger species , 9 Mucor species ,12 Candida Albicans , 8 penicillium , 4 Curvularia species , 4 Aspergillus flavus 4 and all the 11 dermatophytes belonged to the Trichophyton species.

INTRODUCTION

- **INTRODUCTION:**

Fungal infection of the skin, hair and nails are a common public health problem world wide. Cutaneous fungal infections are one of the most prevalent human infections in clinical practice. Skin fungal infections are caused mainly by

dermatophytes.Dermatophytes are a group of closely related filamentous fungi able to damage and utilize keratin found in the skin , hair and nails. Dermatophytosis is an infection produced by dermatophytic fungi in the keratinized tissues. These cutaneous mycoses affect 20%to 25% of the world's population.

Dermatophytes are referred to as tinea infections and can be classified according to the body site involved (1). Tinea infections are found all over the world, but they are specially common in the tropics and in areas with high humidity, over crowding, and unsanitary living conditions. The clinical manifestations and causative types of shallow parasitic infection vary depending on geographic location and financial circumstances (2).

These illness are usually transmitted through direct contact with diseased persons or animals, or through indirect contact with contaminated soil or formites. Dermatophytosis – has become a major public health issue impacting children, adolescents and adults(1). Despite therapeutic advancements in recent decades, the prevalence of utaneous mycoses has continued to rise. Dermatophytosis has been more common during the last two decades, particularly in immuno compromised patients with AIDS, Diabetes, cancer etc. Dermatophytes, yeast and non dermatophyte moulds can all cause cutaneous fungal infections, although dermatophytes are responsible for majority of cutaneous fungal infections(2). Dermatophyte are both keratinophilic as well as keratinolytic. They can infect keratinized tissue in humans and animals, causing dermatophytosis.Trichophyton, Epidermophyton and Microsporum are the three groups of dermatophytes. According to their natural habitat they are classified as anthopophilic,zoophilic, or geophilic. This infection affects both the healthy and the immuno – compromised . Dermatophytosis prevalence ranges from 13 percent to 49 percent, depending on the geographical distribution .Candida albicans, a normal commensal in the mouth and intestine, reaches deeper tissues only when mucosal or cutaneous barriers are breached by disease. Some fungi such as aspergillus are said to be oppurtunists in that they usually infect hosts with compromised immunity(3)

A single species could be involved in a variety of clinical scenario, each with its own pathophysiology. The degree of these reactions is determined by the hosts immunological status as well as the strain and species of the infecting organism. The prevalence of skin fungal infection differs with social, geographic and economical status. So keeping all these factors in mind, the aim of the present study is to isolate and identify dermatophytes from skin, hair, and nail samples through microscopy using potassium hydrochloride (KOH) mount and culture on sabrourand dextrose agar(4)

Many fungal infections develop resistance to antifungal agents in routine use and treatment becomes difficult.

MATERIAL AND METHODS

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AIM

To isolate and identify fungi from samples received in microbiology lab from skin and ENT outpatient department of a tertiary care hospital.

OBJECTIVES

To isolate and identify the fungal strains by phenotypic methods from patients suffering with mycostic fungal infections from skin and ENT outpatient department of a tertiary care hospital

DESIGN OF THE STUDY: Single centre, cross sectional and analytical study.

- STUDY PERIOD: The work was carried out from January 2021 – December 2021
- PLACE OF THE STUDY: Department of Microbiology, central laboratory of Sree Balaji Medical College and hospital a tertiary care hospital in Chennai, Tamilnadu South India.
- COLLABORATING DEPARTMENTS:
- Department of Dermatology
- Department of ENT
- Sree Balaji Medical College and Hospital Chennai- 44

- ETHICAL CONSIDERATION: Approved by Institutional Ethics committee, sree Balaji Medical College and Hospital, Chromepet, Chennai-44. Bharath institute of higher education and research.
- STUDY GROUP: Study group include 100 patients, in the age group 10 – 80 years . Attending skin and ENT outpatient departments with cutaneous and external auditory canal infections presenting with itchy lesions.
- EXCLUSION CRITERIA – Nil
- STUDY SAMPLES:
- From dermatology department – Skin, hair, nails Skin – scrappings, hair plucked, nail clippings and scrappings, hair including hair roots, scalp scrappings.
- From ENT department – Ear swabs from external auditory canal.

METHODS

The study population includes 100 patients with clinically suspected superficial fungal infections, who attend the out patient department of dermatology at Sree Balaji medical College and hospital, Chennai for a period of 4 - 6months . Skin scales, hair and nail samples were collected from patients with cutaneous infections. Direct wet mount microscopic examination was done using 20% potassium hydroxide (KOH) for nail and hair and 10% potassium hydroxide wet mount for skin scrappings. The collected specimens were inoculated into two sets of sabouraud's dextrose agar. The culture tubes were incubated at two different temperature 28 degree and 37degree c. After incubation, culture growth was observed once every two days and the tubes were discarded only after six weeks in the absence of growth. The Microscopic examinations of fungal growth were identified by lactophenol cotton blue stain.

Culture identifications is done based on growth rate, temperature, colony characteristics, colour, texture and pigment production, Dermatophytes were identified depending on nature of mycelium and formation of macro and microconidia of the isolates, budding yeast cells of candida albicans and other candida species were identified by gram stain and LCB mount. Germ tube test was done for the identification of Candida albicans.

For hair and nail examinations, a 20 percent potassium hydroxide (KOH) wet mount was used, and a 10 percent potassium hydroxide wet mount was used for skin scraping. The gathered specimens were inoculated into two different types of fungal culture media: sabourauds dextose agar with gentamicin and cyclohexamide for dermatophytes and sabourauds dextose agar with gentamicin for non-dermatophyte growth

Temperatures of 25°C and 37°C were used to incubate the culture tubes. After incubation, the culture was checked every two days for growth, and the tubes were discarded after six weeks if there was no growth. Growth rate, temperature, colony features, colour, texture, and pigment production were used to identify cultures. For better sporulation and conidating growth, the culture tubes containing mould group of fungal growth were inoculated into dermatophyte test medium and potato dextrose agar. Lactophenol cotton blue stain was used to identify fungal development in microscopic studies. Fungi were identified based on colony morphology, pigment production and on the type of mycelium and the production of macro and micro conidia. Slide culture was used to identify the precise structure of fungus or undisturbed morphological structure in questionable morphological isolates.

SPECIMEN COLLECTION :--

According to the site of infection, skin scrappings, nail fragment, hair fragment were collected from the individual with cutaneous infection. Cotton swab can also we used to collect the specimen, but care has to be taken that swab is swept only in depth of the lesion without touching the adjacent skin margins. It is useful in lesion where there is skin break down. In case of swab, two swabs are taken one is used for bacterial culture and the other one for gram staining.

SPECIMEN TRANSPORT

Immediately following collection, the specimen has to be promptly delivered for microbiological analysis. Any delay is anticipated then storage at room temperature is considered to be appropriate for maintenance of aerobic and anaerobic microorganisms, lower temperature will cause increased oxygen diffusion and raised temperature may cause death or differential growth of some microorganism.

MICROSCOPY

For Candida species,

- Gram stain and culture on SDA . In this budding gram positive yeast cells are seen. Cream coloured , smooth colonies appear within 24hrs.
- **KOH MOUNT**

Budding yeast cells are seen.

REVIEW OF LITERATURE

Fungal infection

Fungus are eukaryote prostista non chlorophyloous organisms with a differentiated nucleus with nuclear membrane and chromosome, rigid cells containing chitin, manan, and Polysaccharide. Their cytoplasmic membrane contains sterol it may be unicellular or multicellular and divide asexually, sexually, or by both(5).

DIFFERENCES OF FUNGI FROM BACTERIA

All fungi are eukaryotic protista that differ from Bacteria and other prokaryotes in many ways:

- They possess rigid cell walls containing chitin, Mannan and other polysaccharides.
- The cytoplasmic membrane contains sterols.
- Cytoplasmic contents include mitochondria and endoplasmic reticulum.
- They possess true nuclei with nuclear membrane and paired chromosomes.
- They may be unicellular or multicellular.
- They divide asexually, sexually or by both processes.
- Most fungi are obligate or facultative aerobes.
- They are chemotrophic, secreting enzymes that degrade a wide variety of organic substrates into soluble nutrients which are then passively absorbed or taken into the cell by active transport.
- The cells show various degrees of specialization.(6)

BENEFICIAL EFFECTS OF FUNGI

- They reside in nature and are essential in breaking down and recycling organic matter
- Some fungi greatly enhance our quality of life by contributing to the production of food and spirits.
- Other fungi have served as medicine by providing useful bioactive secondary metabolites such as antibiotics (e g, penicillin) and immunosuppressive drugs (e g, cyclosporine).
- Fungi have been exploited by geneticists and molecular biologists as model systems for the investigation of a variety of eukaryotic processes.(7)

HARMFUL EFFECTS OF FUNGI

In addition to their disease – producing potential in humans, fungi are directly or indirectly harmful in many other ways. Fortunately, only a few hundred species of fungi have been implicated in human disease, and 90% of human infections by fungi can be attributed to a few dozen species(8). Fungus infections have assumed greater importance with the control of most bacterial infections in the developed countries. For instance, it has been stated that in the USA, fungus infections cause as many fatalities today as whooping cough, diphtheria, scarlet fever, typhoid, dysentery and malaria put together.

PREDISPOSING FACTORS FOR FUNGAL INFECTIONS

- Colonization
- Broad spectrum
- Antibiotics
- Indwelling central catheter
- Total parenteral nutrition
- Immuno suppression
- Burns
- And general measure of severity of illness

GENERAL STRUCTURE OF FUNGI

Fungi grow in two basic forms, as yeasts and molds.

YEAST

The simplest type of fungus is the unicellular budding yeast.

HYPHA

Elongation of the cell produces a tubular , thread like structure called HYPHA.

Hyphae may be septate or nonseptate . Some hyphae are divided into cells by cross-walls or septa, typically forming at regular intervals during hyphal growth(9). The septa, when present , have holes through which free flow of cytoplasmic material can take place. One class of medically important molds, the zygomycetes, produces hyphae that are rarely septated.

MYCELIUM

A Tangled mass of hyphae constitutes the mycelium. Fungi which form mycelia are called molds or filamentous fungi(9) . Under standardized growth conditions in the laboratory, molds produce colonies with characteristic features such as rates of growth, texture, and pigmentation.

In a growing colony of filamentous fungus, the mycelium can be divided into the vegetative mycelium and the aerial mycelium.(10)

VEGETATIVE MYCELIUM

The Hyphae that penetrate the supporting medium and absorb nutrients are the vegetative or substrate hyphae.(11)

AERIAL HYPHAE

In contrast, aerial hyphae project above the surface of the mycelium and usually bear the reproductive structures of the mold.

TYPES OF FUNGI

ACCORDING TO MORPHOLOGY

- Yeast(unicellular) (Cryptococcus)
- Yeast like fungus (candida)
- Filamentus fungi (dermatophyte)
- Dimorphic fungi (blastomysces)

ACCORDING TO SPORE PRODUCTION

- Phycomycetes
- Ascomycetes (penicillium)
- Basidiomyceles (mushroom)
- Fungi imperfecti (most fungi of medical importance)

ECOLOGY OF FUNGI

The fungi are capable of existing and flourishing in a wide variety of environment as parasites, saprobes or symbionts(12)

CHARACTERISTICS OF FUNGI

The fungus is a divorce group of hetrotropic organism that exist as saprobes, commensols or parasites. Most of them are found on decaying vegetative material and in the soil . At an ambient temperature the fungus grows as spore- bearing mold, which is the infectious form to man and animals(12).

MORPHOLOGICAL FEATURES :

Since the fungi are eukaryotic with range of internal membrane system, membrane bound organels and a well-defined cell wall, which are composed largely of Polysaccharide and chitin(13). These show considerable variation in size and shape but can be broadly divided into to main groups,

- A. Yeast
- B. Molds

Yeast

The yeast are unicellular fungal organisms, which reproduce by asexual process known as budding with narrow and broad based budding or by fission. The cell develops protuberance that enlarges and eventually separates from the parent cell(13) The yeast may produce chains of elongated cells known as pseudo hyphae. In some cases they are a phase of growth in the life cycle of a filamentus fungi, which takes place only under specific environmental conditions. The yeast are ubiquitous in the environment being found on fruits, vegetables and other plant materials (exogenous). Some live as normal inhabitants in and or on the human bodies (endogenous). Therefore, these yeast may be found in the clincal specimen as commensols without any medical significance(14)

Molds

The molds are composed of branching filaments known as hyphae. They grow by apical extension, forming an interwoven mass called as mycelium. The hypha is a structural unit of the mycelium(14). In most of the fungi the hyphae have regular crosswalls i.e. Septate. Penicillium and many other fungal genera but in lower fungi these are usually absent i. e. Non -septate Or sparsely septate with wide angel branching as seen in zygomycetes. Similar types of non - septate hyphae are also seen in pythium insidiosum, which is a hydrophilic parafungal organism and Morphologically mimic zygomycetes(16). Hyphae that grow submerged or on the surface of a culture medium or called vegetative hyphae because they are responsible for absorption of nutrients. The hyphae that project above the surface of the medium are called aerial hyphae and produced specialized structures called as conidia(14). The homothallic fungi are self - fertile in which sexual reproduction takes place within the thallus whereas heterothallic fungi are self-sterile and require to compartable thalli fir the sexual reproduction.

CANDIDA ALBICANS

CANDIDIASIS is the commonest fungal disease found in humans affecting mucosa, skin , nails and internal organs of the body. This is caused by several species of yeast – like fungi belonging to genus candida with candida albicans as the representative species(15). The infection may be acute or chronic , superficial or deep and it's clinical spectrum is wide to make a specific definition of the diseases caused by these species. It is found mainly as secondary infection in individuals with some underlying immunocompromised condition and very rarely as a primary disease.

CANDIDA SPECIES

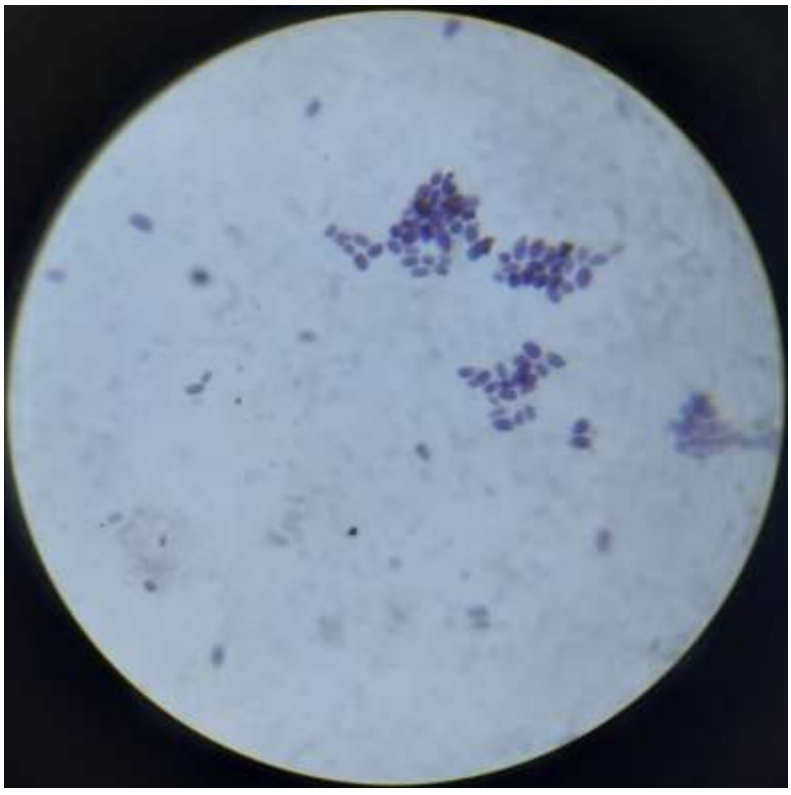

FIGURE - 1

The history of candidiasis is very old as the disease was described in the ancient times and gradually it's etiological agents, diagnostic procedures and therapeutic measures were established(16).The first known description of candidal infections as oral thrush in patients with underlying diseases may be found in Hippocrates ' Epidemics' from the fourth century BC(17). Rosen von Rosenstein and Underwood identified candidal infections in pediatric patients and made the first description of thrush in modern medicine. Bennett isolated the fungus in 1844 from sputum of a patient suffering from tuberculosis(16) Later on it was also isolated from other body sites like vagina, blood and brain by various workers.

Candida albicans is an opportunistic fungal pathogen with host interaction abilities that range from commensal through life-threatening disseminated diseases .The interplay between Candida and host defences is paramount in determining infection outcome. The colonies of this yeast are cream-colored ,pasty and smooth. The rate of growth is rapid and they mature in three days. On cornmeal agar at 25 degree C,psdohyphae are seen in clusters. Large thick- walled, terminal,chlamydospores are the characteristics of the species(18). On CHROMagar Candida,the colonies of C.alblicans appear as light green to bluish-green.

CANDIDIASIS

Candidosis (candidiasis, moniliasis) is an infection of the skin, mucosa, and rarely of the internal organs, caused by a yeast-like fungus Candida albicans, and occasion ally by other Candida species. Several species of the yeast genus candida are capable of causing candidiasis(eg.) Candidosis is an opportunistic endogenous infection, the commonest predisposing factor being diabetes(18). They are members of the normal flora of the skin, mucous membranes, and gastrointestinal tract. Candida species colonize the mucosal surfaces of all humans during or soon after birth, and the risk of endogenous infection is ever-present

MORPHOLOGY

In culture or tissue, candida species grow as oval, budding yeast cells (3-6 um in size). They also form pseudohyphae when the buds continue to grow but fail to detach, chains of elongated cells that are pinched or constricted at the septations between cells and C. albicans is dimorphic (19). In addition to yeasts and it can also produce true hyphae.

SPECIES OF CANDIDA

PATHOGENESIS

Superficial (cutaneous or mucosal) candidiasis.

Superficial (cutaneous or mucosal) Candidiasis It is established by an increase in the local census of candida and damage to the skin or epithelium that permits local invasion by the yeasts and pseudohyphae. The risk factors associated with superficial candidiasis include AIDS, pregnancy, diabetes, young or old age, birth control pills, and trauma (burns, maceration of the skin)(19)

Superficial Candida infections involving the skin, nails and mucous membranes of the mouth and vagina are very common throughout the world. Candida albicans accounts for 80-90% of cases, but other species, notably C. glabrata, C. parapsilosis, C. tropi calis, C. krusei and C. guilliermondii, may occur(20)

On Sabouraud dextrose agar Candida species grow predominantly in the yeast phase as round or oval. cells, 3-8 mm in diameter. A mixture of yeast cells, pseudohyphae and true hyphae is found in vivo and under micro-aerophilic growth conditions on nutri tionally poor media. C. glabrata does not form either hyphae or pseudohyphae(20)

Epidemiology

Candida species, usually C. albicans, are found in small numbers in the commensal flora (mouth, gas trointestinal tract, vagina, skin) of about 20% of the normal population. The carriage rate tends to increase with age and is higher in the vagina during pregnancy(21)

.Lesions caused by candida are as follows:

Mucocutaneous Lesions

1. Oral thrush: Oral thrush can occur on the tongue, lips, gums, or palate. It is found commonly in bottle fed infants and the aged and debilitated. Creamy white patches appear on the tongue or buccal mucosa, that leave a red oozing surface on removal. 2. Vulvovaginitis: Yeast invasion of the vaginal mucosa leads to vulvovaginitis.

After Studying the characters of growth on sabouraud's medium , emulsify small portion of colonies in lactophenol stain solution(21). Cover it with cover slip and study it under microscope and record your findings. Suspend a small inoculum of growth in a small tube containing 0.5ml of sheep serum. Incubate it at 37c for 2 to 3 hours . Remove portion of sediment and place on a slide, cover the slide with cover slip and examine it microscopically for the presence of germ tube.

Growth on sabouraud's medium consisted colonies which are white, smooth , domed , shiny and moist.

Microscopic examination of lactophenol preparation of growth showed yeast cells with budding and pseudohyphae.

Growth inoculated in sheep serum incubated at 37c for 3hours and on microscope examination of portion of it showed germ tube. A germ tube was seen as appendage, one half the width and 3 to 4 times the length of the cell from which it arises.

Colonies' characters, budding yeast cells on microscopic examination means this fungus is candida. The presence of germ tube is characteristic of candida albicans.(21)

It helps in growth characteristics, budding yeast cells, pseudohyphae and positive germ tube production are quite helpful in labelling the fungus as candida albicans.

CLINICAL FEATURES

MUCOSAL INFECTION

This is the most common form of superficial candido sis. Discrete white patches develop on the mucosal surface, and may eventually become confluent and form a curd-like pseudomembrane (21).

In oropharyngeal candidosis white flecks appear on the buccal mucosa, tongue, and the hard and soft palate; although these are adherent, they can be removed. The surrounding mucosa is red and sore. This form of oropharyngeal candidosis occurs most frequently in infancy and old age, or in severely immu nocompromised patients, including those with AIDS(21) Other forms of oral candidosis occuof lesions in the occluded area under the denture in

those who wear dentures painful infection of the tongue in some individuals receiving antibiotic therapy • chronic infection with extensive leucoplakia and infection of the angles of the mouth (angular cheilitis).

In vaginal candidosis, itching, soreness and a non homogeneous white discharge accompany typical white lesions on the epithelial surfaces of the vulva(20). vagina and cervix. Sometimes the mucosa simply appears inflamed and friable. The perivulval skin may become sore and small satellite pustules may appear around the perineum and natal cleft(21). Some women suffer recurrent episodes.

Chronic, intractable oropharyngeal candidosis. which may extend to give oesophageal infection, is common in persons with HIV infection, although the use of combinations of antiretroviral drugs has reduce d its incidence. The appearance of this infection can be the indicator of the transition from HIV positive status to AIDS.

Skin and nail infection

Cutaneous candidosis is less common than dermato phytosis. The lesions usually develop in warm, moist sites such as the axillae, groin and submammary folds. In infants, Candida species are often secondary invad ers in napkin dermatitis(21). Infection of the finger webs. nail folds and nails is associated with frequent immer sion of the hands in water.

Chronic mucocutaneous candidosis

This is a rare form of candidosis that usually becomes apparent in childhood

Candida species

Virulence factors of Candida species

As the antigenic components have been identified immunologically,without emphasis on biological functions of associated molecular species and virulence factors, have been characterized in terms of function without consideration of antigenicity(22). These virulence factors are as follows:

1.Toxins

2.Enzymes

3.Adhesin

4.Complement Receptors

5.Phenotype switching

Toxins: the glycoprotein extracts of Candida cell walls like bacterial endotoxins are lethal and pyrogenic and induce anaphylactic shock I'm various animals models with variable potencies.

DERMATOPHYTES:

Man's cutaneous infections comprise a wide range of illnesses that affect the integument and its appendages, such as the hair and nails. Infection is usually limited to the non-lining cornified layer, although the presence of the infectious agent and its metabolic products causes a range of alterations in the host(22). The Dermatophytes, a homogeneous group of keratophilic fungi, are responsible for the majority of infections. A single species may be implicated in several clinical Types, each with its own pathophysiology. Fungi are man's most frequent infectious agent, and no group of individuals or geographic location is immune to taenia or ringworm infection (taenia-latin for worm). Dermatophytes have evolved towards an accommodating host parasite relationship, which is not seen in other fungal agents that cause human illness. Dermatophytosis is the name given to this group of diseases. Dermatophytes are a group of closely related organisms that utilise keratin as a source of nitrogen. Dermatophytes are classified into three taxa based on clinical, morphologic, and microscopic characteristics: Trichophyton, Microsporum, and Epidermophyton. Dermatophytes can be Anthropophilic (love of humans), Zoophilic (love of animals), or Geophilic (love of the earth) (soil loving). Dermatophytes comes in a variety of clinical forms, depending on the anatomical location and the etiological agent(23). An eczemiform reaction is first induced in the host, followed by allergy and inflammatory symptoms.

MORPHOLOGY:

Chlamydospores and pectinate hyphae are two morphological variations of hyphae. The former are globose bulges encircled by a thick halo wall and located on the hypha's distal section Alternatively, it could be intercalated on its path(23). Pectinate hyphae are hyphae that are pectinate in nature.morphology resembling a comb. Dermatophytes reproduce sexually in their ideal condition, with ascospores being the result of the fusing of two gametes of opposite sign (conventionally gamete + and gamete -). Ascocarps (cleistotethia) are more or less spherical formations that are ringed by thick and claw-like hyphae called peridial.Each ascocarp is made up of sacs (asci), which contain the ascospores of R. Vazquez, J.M. Riesco, and A. Martin Pascual. Mannizzia has been characterised as the perfect condition of the genus Microsporum, and Arthroderma has been described as the perfect form of the genus Trichophyton.(23)

The Dermatophytes have different morphologies depending on whether they are viewed as pathogens obtained directly from lesions (where they are simply seen as unicellular and sporulated forms), b) in artificial culture media (where they develop a reproductor mycelium composed of macro- and microconidia, special hyphae, and other features that have served as the basis for their taxonomic classification), or c) when they are grown using the hair bait technique .(23)

TYPES OF DERMATOPHYTES:

Dermatophytes are a group of closely related filamentous fungi and are classified as,

1. Trichophyton
2. Microsporum
3. Epidermophyton

PATHOGENESIS:

Dermatophytes are not pathogens that are produced by the body. Dematophytes are transmitted in three ways, each with its own set of characteristics(23). Dermatophytes usually infect just the outer cornified layer of the skin, although they can cause significant morbidity. Thiers adaption has developed to a new host, resulting in increased chronicity and infectious dissemination. Unlike most other fungus, Dermatophytes generate keratinase (enzymes that break down keratin), allowing fungi to invade keratinized tissue(23). Dermatophytes have immuno-inhibitory mannans in their cell walls. Mannans also

reduce growth in T.rubrum, lowering the likelihood of the fungus being sloughed off prior to invasion(22). This is considered to have a key role in the chronicity of T.rubrum infection.

DIAGNOSIS IN LABORATORY

Diagnosis in the laboratory is based on the demonstration of microscopy, fungal isolation in culture, and serological assays to determine the causal pathogen in tissue(23).

DIRECT KOH MOUNT

Hair follicles, skin scrapings, and nail scrapings were treated with 20% KOH for 10 minutes, then mounted on a glass slide and examined under a microscope for the presence of fungus at low magnification. The dermatophyte species was isolated from the positive samples using Sabouraud's Dextrose Agar (SDA, Himedia).

ISOLATION OF DERMATOPHYTES

Under sterile conditions, the samples were cultured on Sabouraud's Dextrose Agar (SDA, Himedia) containing Cyclohexamide (0.05%) and chloromphenicol (0.004%). For four weeks, the plates were incubated at 30°C and the growth was monitored. With an L-shaped inoculating needle, dermatophytic growth was scooped up and streaked on SDA slants. The morphology, texture, and pigmentation (obverse and reverse) of the colonies on the slants were all investigated. Microscopic analysis of the stained preparations, as described below, was used to corroborate the results

IDENTIFICATION BY MICROSCOPY

Each isolate's colony was stained with Lactophenol Cotton Blue (LCB) and viewed under a light microscope with low (10 lens) and high power (40 lens) settings. Features such as hyphae arrangement (pencil shaped, spiral, pyriform, septations, etc.), microconidia, and macroconidia were used to make the identification (tear shaped, drop like, spherical, in bunches, abundance or rare etc.). Trichophyton rubrum (ATCC-28188), Trichophyton mentagrophyte (ATCC-18748), and Microsporum gypseum (ATCC-24102) were used as reference strains in the study and were procured from the Post Graduate Institute of Medical Education and Research (PGIMER) Chandigarh.

TINEA PEDIS

This is the infection of the plantar aspect of the foot, toes and interdigital web spaces. The warmth and moisture produced by the shoes are the key factors in establishing and maintaining the infection. It is frequently seen among the individuals wearing shoes for long hours and popularly known as Athletes foot. In the toe webs, scaling , fissuring, maceration and erythema may be associated with an itching or burning sensation. The small vesicles rupture and discharge a thin fluid. Due to the maceration and peeling, cracks appear which are prone to secondary bacterial infections. When secondary infection does occur, lymphangitis and lymphadenitis develop(23). The infection of the sole may extend to the sides of the foot and therefore, it is also known as Moccasin or sandal ringworm.

TINEA UNGUIUM

Tinea unguium is the dermatophyte infection of the nail plates and is largely a disease of adults and is caused by T.rubrum, T.mentagrophytes and E.floccosum. It begins under the leading free edge of the nail plate or along the lateral nail fold and may continue until the entire nail plate and nail bed are infected.

TINEA CORPORIS

This is infection of glabrous (non- hairy) skin of body and may result from extension of infection in the scalp , groin or beard but the term tinea CORPORIS is properly applied to lesions originating on the glabrous skin with exception of scalp , hands , feet , nails and groin. It is also called as tinea glabrata circinata.

TINEA CAPITIS

This is the infection of the shaft of scalp hairs and presents as following clinical types:

- Inflammatory – keroin, favus and agminate folliculitis
- Non – inflammatory – Blackdot, seborrheic dermatitis – like and grey patch

The infected hairs in tinea capitis appear dull and grey. There is breakage of hair at follicular orifice which creates patches of Alopecia with black dots of broken hairs. A patch of alopecia with broken hair and ring formation at the periphery of a patient. The predominant causative fungal species of tinea capitis belong to genus Trichophyton.

PENICILLIOSIS

There are more than 150 known species of the genus Penicillium. Except for infections caused by Penicillium marnefeii, the role other species of Penicillium have in infections of the clinical entity penicilliosis is difficult to confirm.

Pathogenesis

It causes penicillosis, keratitis, otomycosis and rarely deep infections. Penicillium marneffei causes serious disseminated disease with characteristic papular skin lesions in AIDS patients in South-East Asia. Cutaneous lesions and subcutaneous abscesses have been reported.(22).

PENICILLIUM

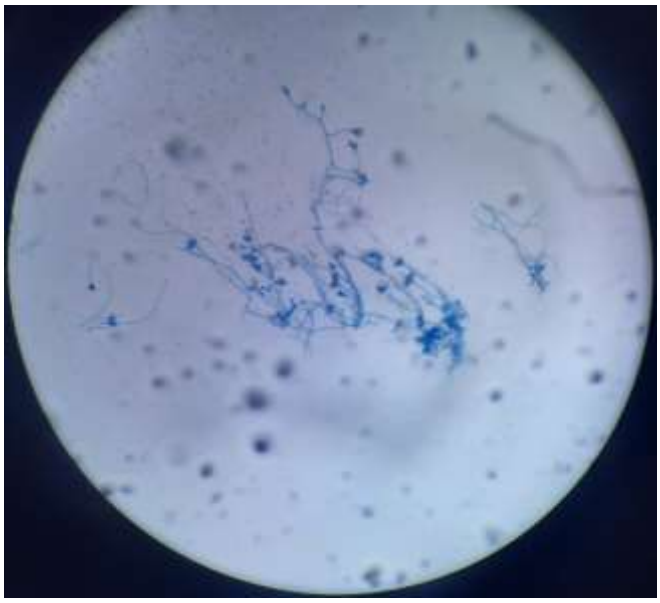

FIGURE - 2

Identification

Fungi belonging to this genus are characterized by pro ducing conidiophores at the tips of branching septate hyphae, which in turn may produce secondary struc tures termed metulae, from which flask-shaped struc tures called phialides bearing smooth or rough- shaped conidia are produced in chains, giving the en tire struc ture a brushlike or broom like appearance(22).

Aspergillus infection

Aspergillus infection in humans were initially described in the mid-1800s. The first attempt to define the genus aspergillus was made by Micheli in 1729 .In the same year he described, named and illustrated the fungus nicely in his famous work, the Nova Geneva Plantarum. He noted that the pattern of condial head of Aspergillus with its spore heads radiating from a central structure, resembled to aspergillus, a brush or perforated globe used for sprinkling holy water hence he named the genus as Aspergillus(23).

ASPERGILLUS

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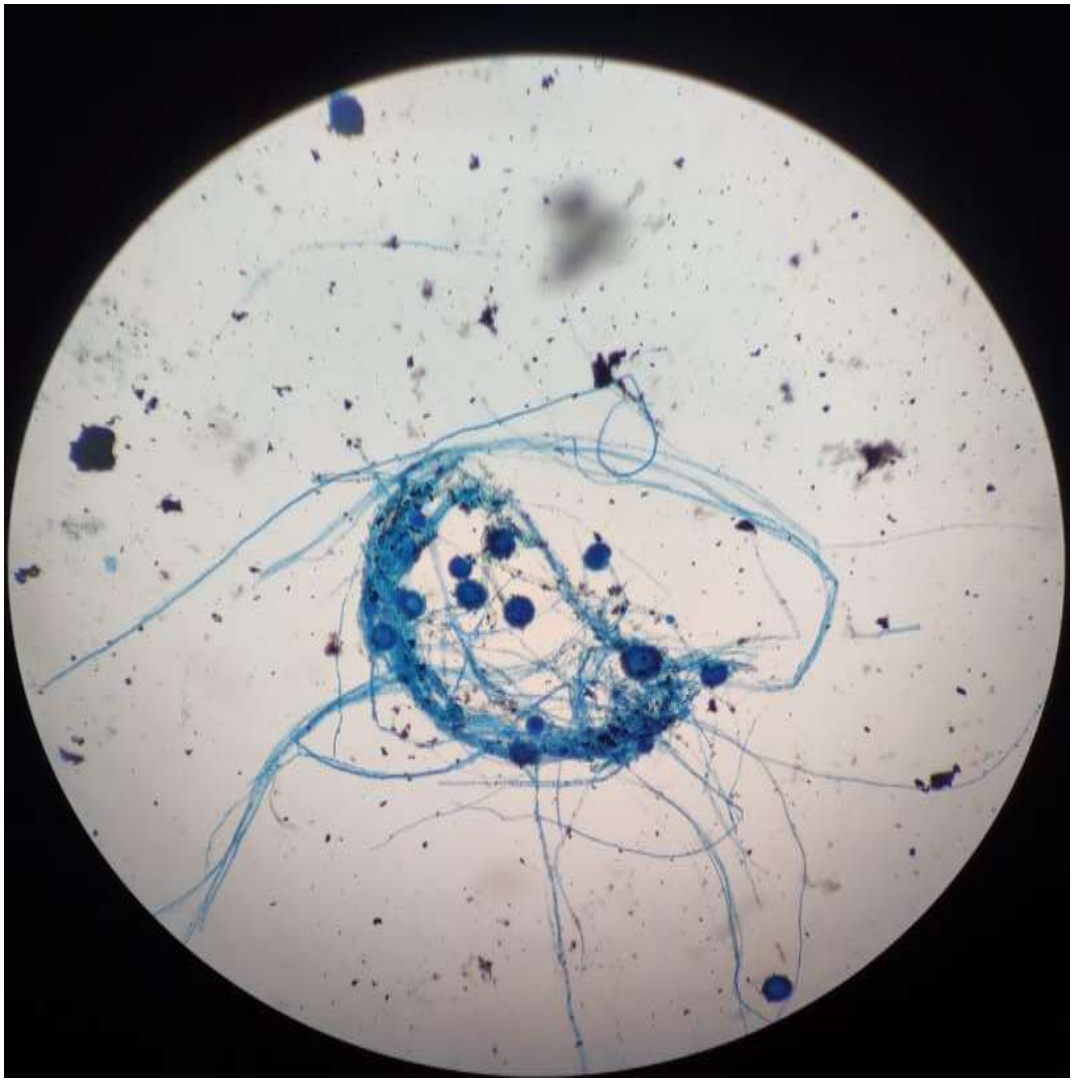


FIGURE - 3

ASPERGILLUS

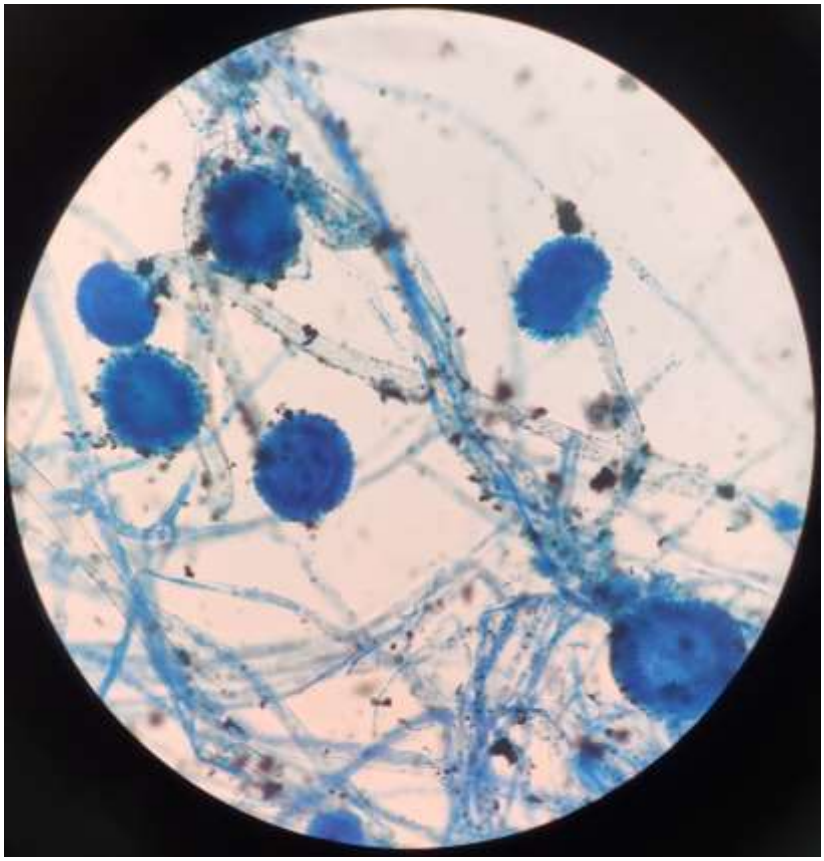


FIGURE - 4

Aspergillosis is a systemic fungal infection found in immunocompromised as well as immunocompetent individuals. This is primarily a pulmonary infection with involvement of other body sites like paranasal sinuses and cutaneous tissues. It is caused by several species of genus Aspergillus, which are ubiquitously found in the environment worldwide in the decaying organic matter(23). In the most serious type of infections,invasion of the lung tissue and dissemination to other organs may take place leading to fatal outcome amoung the immunocompromised patients.

All the Aspergillus species are common saprobic in the soil and on decaying plant material and their spores are ubiquitous in the environment. The human beings are regularly exposed to these spores particularly people working with the decaying vegetarian, like moldy hay in agriculture, are maximally exposed. The disease process generally follows inhalation of the spores but the fungus may also gain direct entry to the body tissues through wounds or during surgery.

Aspergillosis is considered as the second most common fungal infection requiring hospitalization in the United States. The frequency and relative importance of these infections is on the rise in the developed countries, which is possibly related to increased number of immuno compromised patients, owing to improved Survival from AIDS

Aspergillosis is a spectrum of diseases that may be caused by a number of Aspergillus species. Aspergillus species are ubiquitous saprophytes in nature and Opportunistic Mycoses aspergillosis occurs worldwide. There are more than 100 species of Aspergillus but only a few have been implicated in human disease. The most important are: A. fumigatus, A. niger, A. flavus, A. terressand A. nidulans(22).

Pathogenesis

Thismold produces abundant small conidia that are easily aerosolized. Following inhalation of these conidia, atopic individuals often develop severe allergic reactions to the conidial antigens. In immunocompromised patients especially those with leukemia, bone marrow transplant patients, and individuals taking corticosteroids the conidia may germinate to produce hyphae that invade the lungs and other tissues(23).

A. Localized Infections

Localized, noninvasive infections (colonization) by Aspergillus species may involve the nasal sinuses, the ear canal, the cornea, or the nails.

Examples:

Sinusitis A. flarus and A. fumigatus.

Mycotic keratits-A. flavus and A. fumigatus. Otomycosis-Species of Aspergillus particularly A

B. Systemic Aspergillosis

1. Pulmonary Aspergillosis

a. Allergic asthma: In some atopic individuals, devel opment of IgE antibodies to the surface antigens of Aspergillus conidia elicits an immediate asthmatic reaction upon subsequent exposure About 10-20 percent of asthmatics react to A. fumigatus.

b. Bronchopulmonary aspergillosis: In others, the conidia germinate and hyphae colonize the bron chial tree without invading the lung parenchyma. This phenomenon is characteristic of allergic bron aspergillosis, which is clinically chopulmonary recurrent chest infiltrates, defined as asthma, and both type I (immediate) and type III ophilia, in test hypersensitivity to Aspergillus (Arthus) skin antigen. They have difficulty breathing and may antigen develop permanent lung scarring Normal hosts exposed to massive doses of conidia can develop extrinsic allergic alveolitis which follows particularly heavy and repeated exposure to large numbers of spores. A well-known of this form of the disease is Maltsters example of lung, which occurs in workers who handle barley on which A. clavatus has sporulated during the malting process. Colonising aspergillosis (Aspergilloma)

Colonizing aspergillosis usually develops in preexisting pulmonary cavities, such as in tuberculosis or cystic disease. It is also referred to as fungus ball. The fungus grows into large balls (Aspergilloma)(22). Cases of aspergilloma rarely become invasive. Surgical removal becomes necessary as the disease f commonly causes massive hemoptysis.

MUCOR SPECIES

Mucor is a microbial genus of approximately 40 species of moulds in the family MUCORACEAE. Species are commonly found in soil, digestive systems , plant surfaces, some cheeses like Tomme de savoie, rotten vegetable matter and iron oxide residue in the biosorption process.

Colonies of this fungal genus are typically white to beige or grey and fast-growing. Colonies on culture medium may grow to several centimetres in height . Older colonies become grey to brown in colour due to the development of spores. Most of the mucor are unable to infect humans and endothermic animals due to their inability to grow in warm environments close to 37 degrees. Thermotolerant species such as Mucor indicus sometimes cause opportunistic, and often rapidly spreading, necrotizing infections known as zygomycosis. Fungi contain no chlorophyll and most are considered saprophytes. That is, they obtain their nutrition from metabolising non – living organic matter. It occurs in saprotrophs and is most often associated with fungi for example Mucor.

MUCOR SPECIES

FIGURE - 5

IDENTIFICATION OF FUNGI IN SPECIMEN

COLLECTION OF SPECIMEN

- Skin scrap, nails and hair clipping are collected into a piece of clean tissue paper.
- Fold this paper properly and send it to laboratory.
- Sputum , pus, spinal fluid and biopsy of tissues are collected in sterile container.

MICROSCOPIC EXAMINATION

- Sputum , pus , biposy are placed in 10% KOH drop on a clean glass cover.
- Skin scrap, nail clips and hair clips placed into a clean glass slide.
- Add 10% potassium hydroxide solution drop.
- Cover it with cover slip and seal the margin.
- Keep it in an incubator till material is dissolved.
- Examine under microscope to find fungi (yeast, budding yeasts or hyphae) .
- Dried smear is fixed and then gram staining is done

Microscopy

Direct examination of curetted or biopsy material in potassium hydroxide (KOH) may reveal the characteris tic broad, aseptate, branched mycelium and sometimes distorted hyphae. However, they are seen much more clearly when stained with methenamine-silver. The hyphae of these fungi do not stain with PAS. Biopsy is normally the best method of establishing the diagno sis and should be performed early in the course of the infection.

3. Culture the fungi out cycloheximide at 37°C, producing abundant cottony are readily isolated on Sabouraud agar with colonies. Isolation is of little diagnostic significance in the absence of strong supporting clinical evidence

4. Identification Identification is based on the sporangial structures

1. Mucor: Shows nonseptate mycelium without rhizoids (root like structures). Sporangiophores, which may be branched, terminate in large globose sporangia containing numerous spores.

GERM TUBE TEST

The culture of Candida species is treated with sheep or normal human serum and incubated at 37c for 2 to 4 hours . A drop of suspension is examined on the slide under the microscope. The germ tubes are seen as long tube – like projections extending from the yeast cells. There is no constriction at the point of attachment to the yeast cell as seen in case of pseudohyphae. The germ tubes are formed within two hours of incubation in C.albicans and C.dubliniensis. The demonstration of the germ tube is also known as Reynolds – Braude phenomenon.

CULTURAL EXAMINATION

- The scrapping of skin, nail or hair clips, biopsy material , sputum ,etc.are placed on sabouraud's dextrose agar medium (two tubes per specimen).
- Incubate one tube at 37c and the other one at room temperature (22c)
- Incubation is done for 3 weeks.

ANTIFUNGAL SUSCEPTIBILITY TESTING

Antifungal susceptibility testing (measuring the inhibitory activity of the tested antimicrobial agent) and correlations between in vitro susceptibility and clinical outcome of invasive fungal diseases in human patients have been the subject of intensive research. Many factors other than susceptibility of the etiologic agent to the chosen drug affect clinical outcome, including host immune status, location of the infection, duration of the infection, drug pharmacokinetics, and patient compliance.Standardized methods of assessment of in vitro susceptibility of yeasts and filamentous fungi to some common antifungal drugs have been developed. Tentative "breakpoints" for fluconazole, itraconazole, and 5-fluorocytosine against Candida . have been established, and breakpoint values for fluconazole and flucytosine are useful in predicting clinical outcome. Meaningful correlations between in vitro susceptibility test results and clinical outcome for most filamentous fungi and yeasts have not been established.

patients with fungal diseases are to (1) identify a fungal isolate at the genus and species level if possible; (2) perform in vitro susceptibility testing (using approved methods) only for fluconazole and flucytosine susceptibility of Candida isolates from sterile sites; (3) attempt susceptibility testing for Candida spp. and amphotericin B; Cryptococcus neoformans and fluconazole, flucytosine, or amphotericin B; and Histoplasma capsulatum and fluconazole for patients in whom initial antifungal therapy has failed; and (4) select therapy for all other fungal isolates based on guidelines or survey data.

Antifungal sensitivity testing is difficult to obtain in the veterinary clinical setting and results of in vitro susceptibility testing often do not correlate well with clinical response to treatment. In particular, fluconazole may demonstrate low activity with in vitro test systems but high activity in vivo, possibly due in part to the drug's excellent tissue solubility. Currently, determination of fungicidal activities of antimicrobial agents against yeasts and molds holds promise for the development of clinically relevant correlates of in vitro susceptibility.

PREVENTION AND TREATMENT OF FUNGAL INFECTIONS

Fungal infections are treated with antifungal medicines. They have the ability to either kill or prevent fungi from growing and prospering. Antifungal medications are available over-the-counter (OTC) or as prescription prescriptions, and they occur in a range of forms, including:

- ointments or creams
- pills
- powders
- sprays
- Shampoos

.There are several things you can do at home to assist get rid of the fungal infection in addition to using OTC or prescription antifungals. These are some of them:

- cleaning and drying the afflicted area
- garments or shoes that are loose-fitting and enable your skin to breathe
- Make sure to keep your hygiene in check.
- Clothing, towels, and other personal belongings should not be shared.
- Clean your clothes every day, especially your socks and underwear.
- Choose clothing and shoes that allow you to breathe easily. Avoid wearing clothing or shoes that are overly tight or constrictive.
- After showering, bathing, or swimming, make sure to dry off thoroughly with a clean, dry towel.
- Instead of strolling barefoot in locker rooms, wear sandals or flip-flops.
- Wipe down common areas like gym equipment and mats.
- Avoid animals that show signs of a fungal illness, such as missing fur or scratching frequently.

PATHOGENESIS

Fungal infection symptoms.

A fungal skin infection might cause

- Irritation
- Scaly skin
- Redness
- Itching
- Swelling
- Blisters.

TYPES OF FUNGAL INFECTIONS

Fungal skin infections can happen anywhere on your body. Some of the most common are athletes foot, jock itch, ringworm, and yeast infection. The fungi grow best in worm moist places such as shoes, socks, swimming pools, locker rooms and public showers(22). They are often found in the summer and in the hot, humid climates. It happens more often in people who wear tight shoes, who don't change their sweaty socks, and who use public baths and pools.

Fungal skin infection:

Skin is the largest organ of the body which harbours innumerable microorganisms. The epidermis structurally acts as a physical barrier, resisting penetration of microorganism and it's potential toxins while retaining nutrients and moisture inside the body(23). The micro flora tends to occupy stratum corneum and upper parts of the hair follicles. The composition of this flora can vary drastically depending on genetics, sex, climate, age, hygiene, stress, nutrition and hospitalization. The exact mechanism of interaction between the normal micro flora and the humans skin are not well understood but a mutual relationship exists between the flora and the human host. The skin is acidic cool and desicated, but distinct skin habitats are determined by thickness of the skin, folds and density of hair follicles and glands

Fungal Nail infections

- At first, you may only see a white or yellow sopt under your nail.
- Overtime, this spreads and can turn your whole nail white, yellow, green, or black.
- The nail may thick and could be hard to trim.
- It may start to curl up or down or loosen from the nail bed.
- Your nail could become brittle and crumble when you touch it.
- Your nail may become miss shaten.
- You may notice a bad smell.

- It's easy to ignore fungal nail infection at first since you may not have any painpain but if you don't treat them, it can hurt to put any pressure on the area if an infection gets bad enough, it could even become hard to walk.

FUNGAL NAIL INFECTION PREVENTION

- Its a good idea to wash your hands and feet often. Use soap, and make sure you get between your fingers and toes.
- Keep your finger nails and toe nails short and trimmed straight across.
- Wear socks that wick away (absorb) moisture. If your feet sweat a lot, change your socks once or twice a day, or take off your shows snd let your feet cool when you have the chance.

Use antifungal powder or spray on your feet as well as in your shoes. Throw away old pairs of closed -toe shoes since fungi might be living in them.

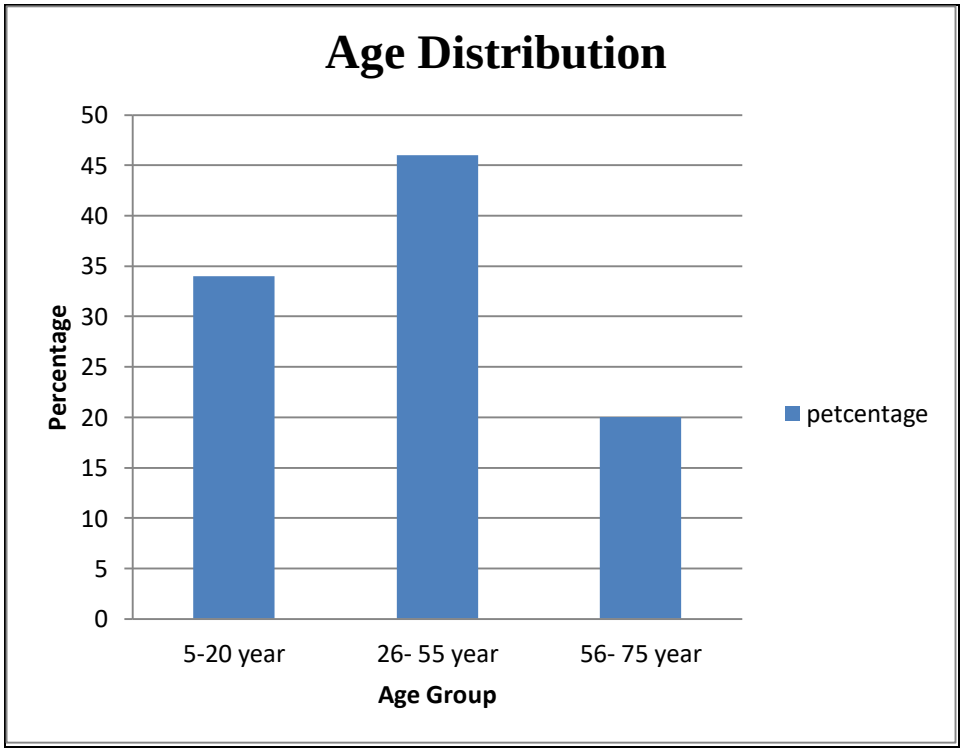
- If you get manicures at nails alone, visit only the ones that disinfect tools after each client. You can also bring your own file and clippers from home. Ask them not to cut your cuticles, since this can cause tiny breaks in the skin that let germs in. **RESULTS**

Age distribution of study samples

TABLE - 1

Age in years	No of samples	Percentage %
5-25 year	34	34
26- 55 year	46	46
56- 75 year	20	20
Total number of samples	100	100

FIGURE - 7

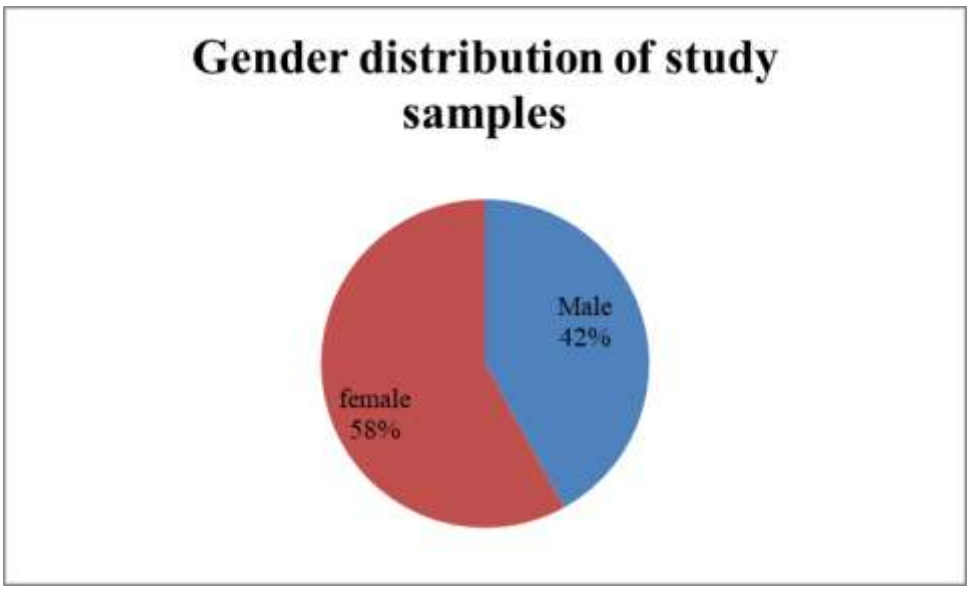


Gender distribution of study samples

TABLE - 2

Total sample	100	100%
Male	42	42%
female	58	58%

FIGURE - 8



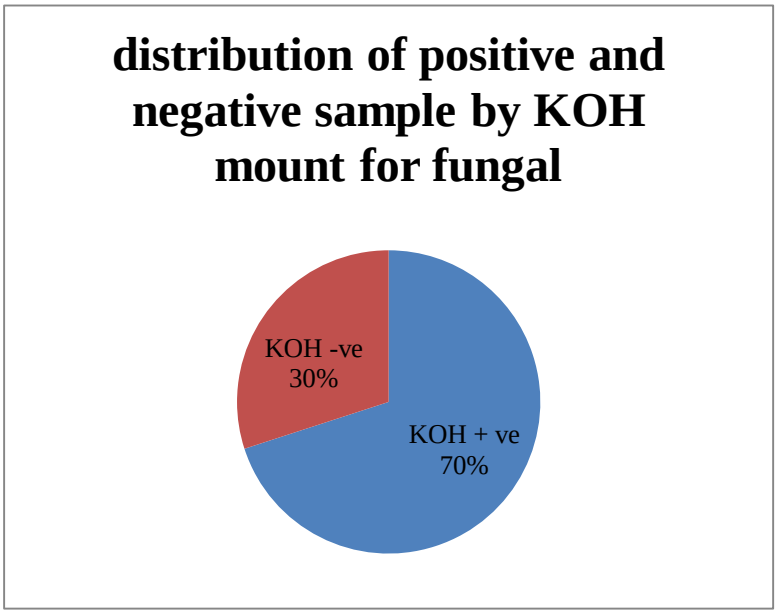
Distribution of positive and negative samples by KOH mount for fungus

TABLE - 3

Total patient sample	KOH + ve	KOH -ve
100	70	30

Out of 100 patient samples , fungal elements were seen in 70 samples

FIGURE - 9



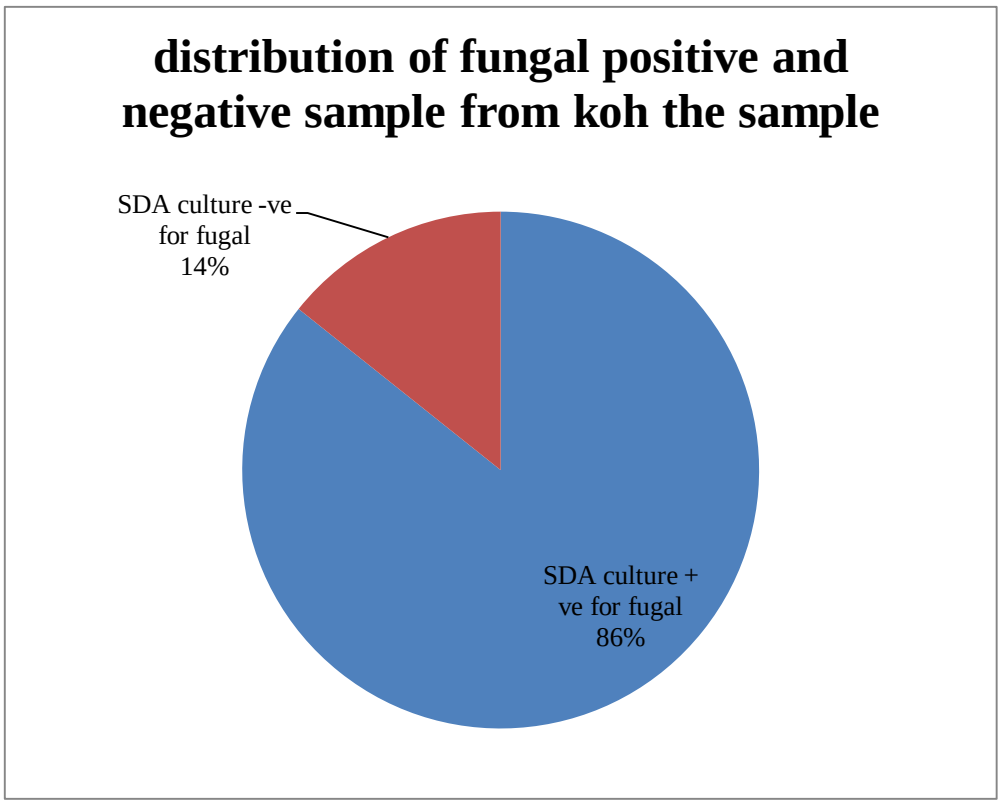
Distribution of fungus positive and negative samples from KOH positive samples

TABLE - 4

TOTAL KOH +ve sample for fungal	SDA culture + ve for fugal	SDA culture -ve for fugal
70	60	10

Out of 70 KOH positive samples, culture for fungus by SDA is seen in 60 samples

FIGURE - 10



Distribution of dermatophytes and non - dermatophytes in SDA culture and LCB mount

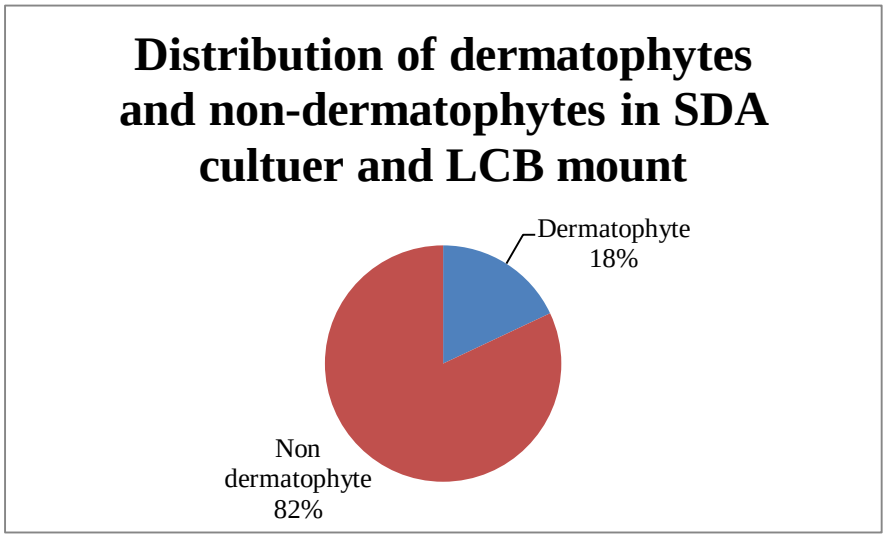
TABLE - 5

Total SDA culture +ve sample	Dermatophyte	Non dermatophyte
60	18	82

Out of 60 SDA culture positive samples, 11(18%) were dermatophytes and 49(82%) were non - dermatophytes (confirmed using lactophenol cotton blue staining).

SDA culture + LCB mount - Dermatophytes + Non - Dermatophytes distribution.

FIGURE - 11

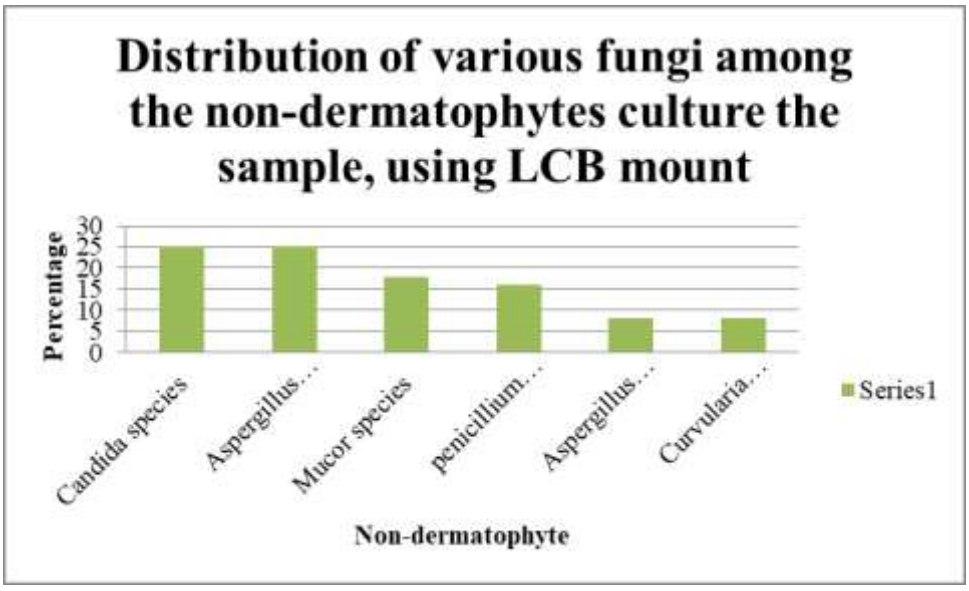


Distribution of various fungi among the non - dermatophytes culture positive samples, using LCB mount.

TABLE - 6

NON- DERMATOPHYTES	100	49
Candida species	25	12
Aspergillus niger species	25	12
Mucor species	18	9
penicillium species	16	8
Aspergillus flavus species	8	4
Curvularia species	8	4

FIGURE - 12



The study population which included 100 patients with clinically suspected superficial fungal infections was subjected to mycological examination. Out of 100 patients 58 (58%) were females and 42 (42%) were males. Maximum cases with infection were between 26 – 55 years of age (46%). Among these age group 17 were males and 29 were females.

Out of 100 specimens from whom the specimens were collected, 40(40%)cases were farmers, 32(32%) cases were construction workers, 28(28%) cases were students and housewives.

Out of total 100 specimens collected 67 (67%) were skin scrappings, 25(25%) were nail clippings, and 8(8%) were from scalp lesions. The maximum number of specimens were skin scrappings, among these 42 specimens were collected from female and 25 from male.

Out of 100 specimens the KOH wet mount was positive for fungal elements in 70(70%) samples and culture positivity was 60(60%). Among these culture positive isolates 49(49%) were non dermatophytes and 11(11%) were dermatophytes,

Among the culture positive 49 non dermatophytes and 11 dermatophytic fungus which includes 12 Aspergillus Niger species , 9 Mucor species , 12 Candida Albicans , 8 penicillium , 4 Curvularia species , 4 Aspergillus flavus and all the 11 dermatophytes belonged to the Trichophyton species .

This study showed among 49 isolates of non dermatophytes and 11 isolates of dermatophytes. Most of the dermatophyte species showed similar pattern of susceptibility to each antifungal agent tested The determinations of susceptible pattern of isolates were identified by high MIC value.

Antifungal drug susceptibility testing for non dermatophyte isolates of Candida species were done .

DISCUSSION

In the present study out of 100 samples processed 60% were females and 40% were males. Female predominant occurs due to their frequent interaction with others, poor personal hygiene and most of them working as farmers and housewives. My study showed that maximum of 49% had non dermatophytes infections in age group between 26-55 years.

My study included 100 patients from whom the specimens were collected. 48% cases did not suffer from the same infection before, 25% cases had previous history of disease, 27% patients had contact history with infected person in their house. Similar findings were seen in study by Suganthi et al(10) who also had shown 49% cases did not suffer from the same infection before which was correlated with my study.

Tinea corporis was the commonest lesion accounting for 15% of cases in my study followed by tinea capitis 10%. This observation correlated with nawal p, Patel et al(11) which showed tinea corporis 40.8% followed by tinea cruris 27% cases. Among the tinea corporis infections most of the clinical condition include lesion in exposed part of the human skin. Similar findings have been shown by Venkatesan et al(21).

Agarwalla et al(13) and grando et al(14) conducted a study on clinico mycological study of dermatophytosis observed more than 20% of patients had two or more clinical types but my study did not show patients with dual clinical presentation which indicates no one had any risk factors for extensive tinea infections.

My study observed KOH mount positive for fungal elements in 70% cases which correlated with Singh s and beena PM (15) who had shown KOH mount positivity as 61%.

The pattern and isolation rate of non dermatophytic fungi obtained in my study was comparable with N dako JA et al(17) showed that out of 100 specimens 16 non dermatophyte fungi were isolated which includes 6 isolates of Candida species which had correlated with my study and 10 isolates of aspergillus species which did not correlate.

Matnani G.et al(22) who had isolated total of 27 candida albicans and antifungal susceptibility testing was done by disc diffusion method by using antifungal drug fluconazole. All the isolates were sensitive in this study which correlated with my study.

SUMMARY AND CONCLUSION

SUMMARY

The study was done for, Isolation and Identification Of Fungi From Samples Received In Microbiology Lab From Skin And ENT Outpatient Department Of A Tertiary Care Hospital In South India.

* The Samples (100) were collected from the Skin and ENT Outpatient department of Sree Balaji Medical & Hospital , Chrompet , Chennai for a period of 6 months (ie)July to December 2021.
* Out of 100 patients samples tested for fungal elements by KOH mount and microscopy, 70 were positive and 30 negative for fungus.
* Out of 70 KOH positive samples, 60 showed growth in SDA culture for fungus.
* Out of 60 SDA culture positive samples 49(82%) were non - dermatophytes and 11(18%) were dermatophytes.
* Out of 49 non - dermatophyte culture positive samples 12(25%) were Candida species , 12(25%) were Aspergillus niger , 9(18%) were Mucor species, 8 (16%) were Penicillium species , 4(8%) were Aspergillus flavus and 4(8%) Curvularia.
* This study clearly shows that fungal infections in tropical countries like India We need to advise patients to take proper care of skin , hair and external auditory canal to prevent fungal infections. Recurrent fungal infections are difficult to treat with topical antifungal applications and may need systemic antifungal medications for total cure , after antifungal susceptibility testing.

CONCLUSION

This study clearly shows that fungal infections are a common cause of skin and ear infections in tropical countries like India. One has to take proper care of skin, hair , nails and ears and maintain good personal hygiene to prevent recurrent fungal infections which can be difficult to treat with topical antifungal agents and may need systemic antifungals (after antifungal susceptibility testing) for cure.

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